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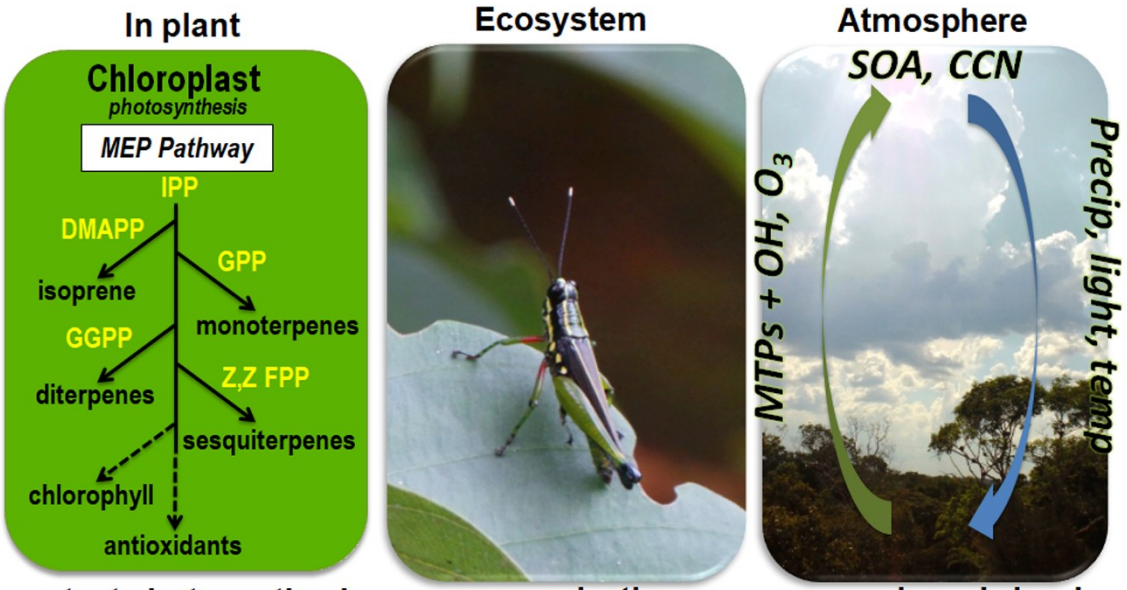

Graphical abstract: Graphical illustration of the biochemical, ecological, and atmospheric roles of volatile isoprenoids (isoprene and monoterpenes) within plants, ecosystems, and the atmosphere. Volatile isoprenoids protect photosynthesis during abiotic stress, are involved in multi-trophic interactions within ecosystems, and following atmospheric oxidation, impact climate through influences over secondary organic aerosol (SOA) and cloud condensation nuclei (CCN) lifecycles in the troposphere.

Leaf isoprene and monoterpene emission distribution across hyperdominant tree genera in the Amazon basin

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Abstract

Tropical forests are acknowledged to be the largest global source of isoprene (C_5H_8) and monoterpenes ($C_{10}H_{16}$) emissions, with current synthesis studies suggesting few tropical species emit isoprenoids (20-38%) and do so with highly variable emission capacities, including within the same genera. This apparent lack of a clear phylogenetic thread has created difficulties both in linking isoprenoid function with evolution and for the development of accurate biosphere-atmosphere models. Here, we present a systematic emission study of "hyperdominant" tree species in the Amazon Basin. Across 162 individuals, distributed among 25 botanical families and 113 species, isoprenoid emissions were widespread among both early and late successional species (isoprene: 61.9% of the species; monoterpenes: 15.0%; both isoprene and monoterpenes: 9.7%). The hyperdominant species (69) across the top five most abundant genera, which make up about 50% of all individuals in the Basin, had a similar abundance of isoprenoid emitters (isoprene: 63.8%; monoterpenes: 17.4%; both 11.6%). Among the abundant genera, only *Pouteria* had a low frequency of isoprene emitting species (15.8% of 19 species). In contrast, *Protium*, *Licania*, *Inga*, and *Eschweilera* were rich in isoprene emitting species (83.3% of 12 species, 61.1% of 18 species, 100% of 8 species, and 100% of 12 species, respectively). Light response curves of individuals in each of the five genera showed light-dependent, photosynthesis-linked emission rates of isoprene and monoterpenes. Importantly, in every genus, we observed species with light-dependent isoprene emissions together with monoterpenes including β-ocimene. These observations support the emerging view of the evolution of isoprene synthases from β-ocimene synthases. Our results have important implications for understanding isoprenoid function-evolution relationships and the development of more accurate Earth System Models.

Keywords: *Protium*, *Licania*, *Inga*, and *Eschweilera,* isoprene and monoterpene emissions in the Amazon rainforest, isoprene synthase, mycene/ocimene synthase

1. Introduction

The photosynthetic uptake of atmospheric CO_2 by the Amazon forest in South America and the photosynthetically-derived emissions of the volatile isoprenoids isoprene (C_5H_8) and monoterpenes ($C_{10}H_{18}$) represent the single largest terrestrial sink of CO_2 and source of reactive alkenes in the global atmosphere (Chambers et al., 2014;

Guenther et al., 1995; Guenther et al., 2006; Jardine et al., 2015). Recent studies have shown that neither isoprene nor monoterpenes are stored in tropical leaves. Emissions are dependent upon biosynthesis linked with the photochemical production of reducing equivalents (NADPH) and energy (ATP) and carbon skeletons derived from the Calvin-Benson cycle (G3P) (Jardine et al., 2014; Jardine et al., 2017). Due to its vast area, high species diversity, and long growing season, the Amazon forest in South America is responsible for an estimated 15% of global terrestrial photosynthesis (Malhi et al., 2008). It is also consistently reported as highly sensitive to climate change variables, such as warming and altered precipitation patterns. Regional-scale tropical forest decreases in gross primary productivity associated with high temperature and drought are increasing in the tropics (Laan-Luijkx et al., 2015; Lewis et al., 2011; Phillips et al., 2009; Zeng et al., 2008), but the key biochemical and physiological mechanisms by which tropical trees defend themselves from these factors are still under debate. One of the earliest processes in plant response to abiotic stress is the rapid accumulation of reactive oxygen species (ROS) that initially function as warning signals that activate defense responses before triggering programmed cell death under excessive ROS accumulation (Petrov *et al*., 2015). ROS signaling is linked to the production and emission of volatile isoprenoids, including isoprene and monoterpenes, which play important roles in minimizing ROS accumulation in leaves through antioxidant mechanisms (Vickers *et al*., 2009). These mechanisms can include the consumption of excess photosynthetic energy and reducing equivalents during isoprenoid biosynthesis (Jardine et al., 2016b), direct ROS-isoprenoid antioxidant reactions (Jardine et al., 2012), and signaling properties of isoprenoid oxidation products (Karl et al., 2010). In addition, volatile isoprenoids can partition into phospholipid membranes, potentially increasing adhesion forces and maintaining stability without changing their dynamic properties. Reinforced hydrophobic interactions within the thylakoids under

abiotic stress are hypothesized to stabilize lipid-lipid, lipid-protein and protein-protein interactions in photosynthetic membranes (Sharkey and Singsaas, 1995).

Despite isoprenoid and other defense mechanisms, if the intensity and duration of abiotic stress is extended over a certain threshold, ROS production will overwhelm the scavenging action of the plant antioxidant system. Extensive cellular damage can result, including membrane peroxidation and the reduction of ecosystem gross primary productivity (GPP), with a shift from terrestrial sinks to sources of atmospheric CO_2. Such a shift in tropical forest carbon balance would eliminate a critical ecosystem service and accelerate global warming (Brienen et al., 2015). Recent observations in the central Amazon have demonstrated unprecedented canopy temperatures during the dry season. Mid-day values can reach over 40 °C (Jardine et al., 2017). Climate models consistently predict warmer conditions in the Amazon Basin by the end of the 21st century (Olivares et al., 2015), including a higher frequency and greater intensity of large-scale Amazonian droughts (Nobre and Borma, 2009; Zeng et al., 2008). Therefore, climate change factors, including warming trends and droughts threaten the ability of tropical ecosystems to maintain a net carbon sink throughout the 21st century, and therefore mitigate anthropogenic climate effects in the atmosphere. Thus, there is an urgent need to better understand the biochemical and physiological mechanisms underlying forest drought response, and in particular the distribution of volatile isoprenoid emissions as defense compounds contributing to thermal tolerance of photosynthesis across diverse tropical forests.

While terrestrial ecosystems in the tropics cover only ~18% of Earth's land surface, they dominate volatile isoprenoid emissions globally (Guenther et al., 2006). For example, isoprene and monoterpene emissions from tropical forests are estimated to account for 88% and 83% of the total global emissions of these compounds, respectively

(Sindelarova et al., 2014). Therefore, it is clear that landscape scale isoprene and monoterpene emissions are highest in the tropics and decrease with increasing latitude (Acosta Navarro et al., 2014). Thus, tropical regions are global hotspots of isoprene and monoterpene emissions due to (i) the high biomass densities and rates of gross primary productivity and (ii) the high light intensities and leaf temperatures that stimulate high leaf emission rates (Alves et al., 2014; Jardine et al., 2014; Jardine et al., 2016b). Even so, tropical ecosystems correspond to a small portion of studies related to volatile isoprenoid emissions, most of which have been performed in temperate regions (Harley et al., 2004). Thus, tropical forest isoprenoid emissions are primarily based on a few limited-duration above-canopy measurements, (Harley et al., 2004; Kesselmeier and Staudt, 1999; Niinemets et al., 2011). Thus, the mechanistic basis for predicting volatile isoprenoid emissions in tropical forests still remains based primarily on temperate forest studies (Guenther et al., 2012). This is due, in part, to logistical, technological, and environmental challenges of working in the tropics and the extremely high tree species diversity. For example, the Amazon forest has been estimated to have anywhere between 6,727 (Cardoso et al., 2017) to more than 16,000 distinct tree species (Ter Steege et al., 2013). While current synthesis studies suggest that 20% of tropical species emit isoprene (Loreto and Fineschi, 2015), systematic studies across the hyperdominant tree genera, which account for a large fraction of individuals in the Basin, have not occurred. As such, one of the major uncertainties in global model estimates of terrestrial isoprene emissions from tropical ecosystems relate to the identity and distribution of species (i.e., plant functional types) that are responsible for isoprenoid emissions in diverse tropical forests.

In this study, using high sensitivity analytical systems for leaf volatile emissions coupled to a portable photosynthesis system deployed to the Amazon forest throughout 2014-2016, we carried out a systematic survey aimed at characterizing light-dependent

emissions of foliar isoprenoids across species in the top five most abundant genera in the Amazon Basin. The core dataset includes controlled light response curves of leaf gas exchange and isoprenoid emissions across five highly abundant tree genera (*Protium*, *Licania*, *Inga*, *Eschweilera* and *Pouteria*) in four established field sites from central to eastern Amazonia. This core data-set is supplemented by additional photosynthesis and leaf isoprenoid emission measurements under standard environmental conditions, as well as qualitative isoprenoid emission measurements without environmental control or supporting photosynthesis observations. The results are discussed in terms of a potential common phylogenetic thread linking isoprenoid function under abiotic stress with evolution and the potential for the improvement of global models linking isoprenoid emissions with atmospheric chemistry and their associated biosphere-atmosphere feedbacks.

2. Results

In total we sampled 162 trees, belonging to 113 different species distributed across 25 botanical families. Many of these species are of great importance for the Amazon region, such as the hyperdominant *Euterpe precatoria* Mart. (Arecaceae), *Eschweilera coriacea* (DC.) S.A.Mori (Lecythidaceae), *Trattinnickia burserifolia* Mart. (Burseraceae), *Socratea exorrhiza* (Mart.) H. Wendl. (Arecaceae), *Protium heptaphyllum* (Aubl.) March. (Burseraceae) and *Licania heteromorpha* Benth. (Chrysobalanaceae). These species are among the 20 most abundant in the Amazon Basin and Guiana Shield, with an estimated population of more than 3.7×10^8 individuals each, according to ter Steege et al. (2013). Of the total 113 species sampled, 61.9% emitted isoprene and 15% emitted monoterpenes. In addition, in 9.7% of species, emissions of both isoprene and monoterpenes were detected (**Table 1**).

Within the collected database of the present study, 69 species (represented by 88 trees) account for the five most abundant tree

genera in the Amazon *Protium*, *Licania*, *Eschweilera*, *Inga* and *Pouteria*. Among the 69 hyperdominant species sampled, isoprene emissions were detected in 63.8% and monoterpenes in 17.4% of the species. In 11.6% of these species both isoprene and monoterpene emissions were observed. Thus, when compared to the total species average (113 species), the abundance of isoprene and monoterpene emitting species within the 5 most abundant tree genera (69 species) was similar. However, when each individual genus was analyzed separately (**Fig. 2**), only species in the *Pouteria* genus showed a low abundance of isoprene and monoterpene emitters (15.8% emitted isoprene and 10.5% emitted monoterpenes). In contrast, the species richness of isoprenoid emitters was found to be exceptionally high in *Eschweilera*, *Inga, Protium*, and *Licania*. For example, 83.3% of the 12 *Protium* species, 61.1% of the 18 *Licania* species, 100% of the 8 *Inga* species, and 100% of the 12 *Eschweilera* species were found to emit isoprene. Isoprene and monoterpenes were observed to occur simultaneously in at least one species within each of the abundant genera, with the exception of *Pouteria*.

It should be noted that given the focus on species, only one measurement was collected from a single individual for the majority of species. However, as summarized in the ‘statistics’ tab of the supplementary database file (Database_S1.xlsx), many species had biological replicates within the same site and sometime across sites. For example, in the Arecaceae family, all 5 individuals of *Manicaria saccifera* Gaertn., all 6 individuals of *Mauritiella aculeata* (Kunth) Burret, all 4 individuals of *Oenocarpus bacaba* Mart., and all 5 individuals of *Socratea exorrhiza* (Mart.) H.Wendl. showed light-dependent isoprene emissions. In the Burseraceae family, all species with multiple individuals studied showed isoprene emissions including *Protium decandrum* (Aubl.) Marchand (2), *Protium hebetatum* Daly (3), as well as unidentified *Protium* species (5). Both *Eschweilera wachenheimii* (Benoist) Sandwith (Lecythidaceae) individuals in forest transects near Manaus, Brazil showed isoprene emissions, as did both

individuals of *Inga edulis* Mart. (Fabaceae), *Couepia guianensis* Aubl. (Chrysobalanaceae), *Vismia guianensis* (Aubl.) Pers. (Hypericaceae), *Scleronema micranthum* Ducke (Malvaceae), *Eperua glabriflora* (Ducke) R. S. Cowan (Fabaceae), and three individuals of *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. (Malvaceae). Both *Cecropia sciadophylla* Mart. (Urticaceae) individuals showed light-dependent monoterpene emissions and both *Licania heteromorpha* Benth. (Chrysobalanaceae) individuals near Manaus and the Caxiuanã National Forest showed isoprene emissions. Likewise, species that were identified to be non-emitters of volatile isoprenoids showed no detectable emissions in multiple individuals studied such as *Pouteria reticulata* (Engl.) Eyma (Sapotaceae), *Licania heteromorpha* Benth. (Chrysobalanaceae), *Chamaecrista xinguensis* (Ducke) H. S. Irwin & Barneby (Fabaceae), *Trichilia* sp. (Meliaceae), and *Virola* sp. (Myristicaceae) However, in some cases, not all of the biological replicates showed consistent isoprenoid emission patterns such as *Eschweilera grandiflora* (Aubl.) Sandwith (Lecythidaceae) and *Pouteria erythrochrysa* T. D. Penn. (Sapotaceae) (1 with isoprene and 1 without detectable emissions), *Scleronema micranthum* Ducke (Malvaceae) (1 with monoterpenes and 1 without detectable emissions), *Pouteria anomala* (Pires) T. D. Penn. (Sapotaceae) (1 with monoterpenes and 2 without detectable emissions). Nonetheless, these species were designated as an emitting species, as low photosynthetic rates were often associated with the lack of isoprenoid detection.

For each of five abundant genera (*Eschweilera*, *Inga, Protium*, *Licania,* and *Pouteria*), light response curves were performed on several or in some cases all of the species in order to demonstrate the strict connection of volatile isoprenoid emissions with photosynthesis. In all, 47/62 individuals were observed to show significant isoprenoid emissions with classic light-dependent patterns (see data in brief companion article for complete dataset, Jardine et al., 2020a). Together with leaf gas exchange data, an example light response curve is shown for one species in each of the 5 hyperdominant genera

(**Fig. 3**). In the dark, photosynthesis is negative due to leaf respiration, and isoprene and/or monoterpene emissions are undetectable. With increasing light intensity, photosynthesis and isoprenoid emissions increase together, although in a non-linear fashion. At low light intensities, photosynthesis increases sharply while isoprenoid emissions increase only moderately. As light further increases, photosynthesis begins to saturate while isoprenoid emissions continue to increase. This pattern results in an increased percentage of photosynthesis being emitted as isoprenoids as light intensities increase.

We observed the presence of extensive microbial leaf surface coatings in the lower to mid canopies which greatly reduced photosynthesis rates and any associated volatile isoprenoid emissions (data not presented). We also observed that when taking branch cuttings from the upper canopy, larger branches (0.5-1.0 m) recut under water on the ground were required in order to achieve high rates of photosynthesis and isoprenoid emissions once the branch was re-established on the ground. Leaves from small branches often did not respond well during the light response curves, a potential consequence of xylem embolism. In addition, we observed that leaves required sufficient time to adapt to their new environment in the leaf chamber, with our light curves providing ample time (1 hour) for the stomata and associated physiology to respond to the new environmental conditions. Thus, false negatives may be obtained if emissions are not evaluated from sunlit upper canopy, using equipment unable to detect emissions of 1 nmol m^{-2} s^{-1} or less, and from fast measurements without giving the gas exchange physiology time to equilibrate.

3. Discussion

The capacity to plants to emit leaf isoprenoids has been previously observed as highly variable, including within the same genera (Fineschi et al., 2013; Kesselmeier and Staudt, 1999). Roughly 20-

317 38% tropical plants are assumed to emit isoprene (Harley et al., 2004;
318 Loreto et al., 2014). Both high and non-isoprene emitters have been
319 reported within the same genus and this apparent lack of a clear
320 phylogenetic thread has created difficulties linking isoprenoid function
321 with evolution and the development of accurate biosphere-
322 atmosphere models.
323 Previous leaf surveys in the tropics have been limited in
324 duration and extent, lacked the capabilities to quantify both isoprene
325 and monoterpenes, lacked a high sensitivity system capable of
326 detecting isoprene emissions below 1.0 nmol m^{-2} s^{-1}, required the
327 shipment of samples internationally for analysis with long associated
328 sample storage times of several weeks or more, and were often not
329 linked with photosynthesis measurements to verify active leaf
330 physiology. Other challenges are the use of shade leaves more
331 accessible to the ground and random species sampling,
332 unrepresentative of the forest. It is recognized that isoprenoid
333 emission capacity is greatly reduced in the understory or shade
334 adapted leaves (Harley et al., 1997). As described in the data in brief
335 and *MethodsX* manuscripts (Jardine et el., 2020a,b), this study
336 addressed these issues by developing a new portable field sampling
337 method and establishing a volatile metabolomics laboratory at the
338 National Institute for Amazon Research (INPA) in Manaus, Brazil.
339 Results from the light response curves showed maximum isoprenoid
340 emission rates always occurred at the highest light intensity (PAR:
341 2000 µmol $m^{-2}s^{-1}$) (**Fig. 3**) with leaf isoprenoid emissions ranging from
342 0.2-44 nmol m^{-2} s^{-1} (**Fig. 4a**). Thus, slow light response curves (e.g. 70
343 min) allow time for the physiology to adapt to the increasing light,
344 often resulting in high rates of photosynthesis and isoprenoid
345 emissions. Given that species showing low isoprenoid emissions were
346 more common than high emissions (**Fig. 4b**), we recommend that
347 future studies employ both the slow light response curves coupled
348 with photosynthesis and a high sensitivity system capable of
349 detecting isoprenoid emissions < 1.0 nmol m^{-2} s^{-1}.

In contrast to other studies that found isoprenoid emissions to be relatively rare in tropical forests and variable across individual genera, we found high consistency of species within abundant genera to emit isoprene in the Amazon. Some species emitted both isoprene and monoterpenes, while a smaller percentage of species emitted only monoterpenes. We found that 4 out of 5 hyperdominant genera had widespread isoprene emissions across representative species.

Of the limited leaf-level studies on volatile isoprenoid emissions in the tropics, a recent analysis compiled existing inventories and estimated that roughly 20% of tropical and temporal plant species emit isoprene (Loreto and Fineschi, 2015). Consistent with this result, in Panama 51 tropical species were surveyed with 29% found to emit isoprene (Keller and Lerdau, 1999). However in a tropical forest in Costa Rica, 10 of the 20 species surveyed showed significant isoprene emissions suggesting that tropical forests may contain a higher fraction of isoprene emitters (50%) than temperate forests (Geron et al., 2002). When a larger survey in the Brazilian Amazon utilizing numerous techniques was compiled consisting of 125 species, 38% were found to emit isoprene (Harley et al., 2004). It should be noted that in the three tropical surveys in Panama, Costa Rica, and Brazil, monoterpene emissions were not evaluated. Moreover, these studies involved largely random sampling of species rather than a systematic survey targeting specific species with enhanced distribution within the forest. Given the extremely high number of estimated tropical species, random sampling of isoprenoid emissions may not produce data representative of the forest. However, depending on the methods used, random sampling strategies could select by chance the most frequent species; Each species is not equally abundant with the abundance heavily skewed towards “hyperdominant” species. In the Amazon for example, it was suggested that just 227 hyperdominant species were so common that they accounted for half of all trees in the forest while accounting for 1.4% of total species (ter Steege et al., 2013). Of the total 752 plant genera in the Amazon

forest, the 5 genera that we targeted in this study (*Eschweilera*, *Protium*, *Pouteria*, *Licania* and *Inga*) were found to represent an estimated 18.7% of the total of individuals in the Amazon (ter Steege et al., 2013). Heterogeneity of tree species across the landscape due to changes in topography also can lead to heterogeneity in emissions across the landscape (Batista et al., 2019).

The results showed much higher percentages of species and individuals emitting isoprene compared to monoterpenes (**Fig. 2**). This result agrees with other studies on emissions of these compounds by plants, which have concluded that monoterpene emissions at the ecosystem scale in broadleaf forests is roughly 10% that of isoprene emissions (Fineschi et al., 2013; Guenther et al., 2012; Sindelarova et al., 2014). Some authors have suggested that isoprene is emitted at higher rates by fast-growing woody plants in early and mid-successional forests, and that monoterpenes are more characteristic of forests in more advanced stages (Fineschi et al., 2013; Harrison et al., 2013). However, this is not consistent with our findings in mature forests in the Amazon where monoterpene emissions were found to be relatively rare whereas isoprene emissions were found to be very common.

Due to its rapid volatilization, it was initially believed that isoprene would not be produced in conjunction with monoterpenes. Monoterpenes were assumed to be only stored in storage structures as resins in plants. Harrison and colleagues (2013) suggested that species with isoprene synthase will preferentially emit isoprene, to the detriment of monoterpenes, and in those species that emit both compounds there is competition between precursors and reducing power. Although monoterpenes are prevalent in stem storage resins of tropical trees in the Amazon (Piva et al., 2019), recent studies using $^{13}CO_2$ have demonstrated that leaf emissions in the tropics do not derive from storage resins. Instead, they derive from biosynthesis linked with photosynthesis as a carbon source like isoprene (Jardine et al., 2017). In the present study, we observed that 9.7% of the total

species studied emitted both isoprene and monoterpenes. This demonstrates that isoprene emissions do not exclude the ability of a species to also produce photosynthetically linked monoterpenes. These dual emitters may provide deep insights into evolutionary histories and functional traits of both isoprene and monoterpenes. Some studies have suggested that the ability to emit isoprene may have been acquired and lost several times throughout plants evolution (Dani et al., 2014; Monson et al., 2013). When lost, it was hypothesized that it would give rise to lower volatility compounds, such as monoterpenes, for better adaptation to repeated and prolonged stress events. However, it was recently suggested that isoprene synthase, the key enzyme responsible for the formation of isoprene in the chloroplast, evolved in close relation with the monoterpene synthase enzyme (i.e., myrcene/ocimene synthase) (Sharkey et al., 2013). Our findings support this hypothesis as we observed numerous species that have significant leaf ocimene and myrcene emissions in the Amazon (e.g. **Fig. 3f**). Moreover, while plants are generally assumed to emit either isoprene or monoterpenes, we observed a species in each of the 5 genera which emitted both isoprene and cis-β-ocimene (e.g. **Fig. 3c**).

Sharkey and Monson (2017) pointed out that it is not yet fully understood how, throughout evolution, the process of isoprene loss and maintenance of this capacity in plants occurs. Our findings suggest that isoprene evolutionary history in trees cannot be addressed without an understanding of its distribution among hyper-diverse tropical forests and cannot be studied in isolation from myrcene/ocimene emissions. Thus, it is necessary to evaluate the connections and interdependencies between isoprene and monoterpene in order to reconstruct accurate evolutionary histories of volatile isoprenoids.

When compared to previous studies on tropical isoprene and monoterpene emissions, the results of this survey stands in contrast with a recent synthesis review. Loreto and Fineschi (2015) suggested

that about 20% of the tropical species are isoprene emitters. The high percentage of isoprene emitting species (74-100%) in the highly abundant Amazon genera (*Protium*, *Licania*, *Inga*, and *Eshweilera*) observed in this study implies that their production is linked with their widespread distribution. In contrast with these values, for the genus *Pouteria* we found only 15.8% of the species emitted isoprene and 10.5% emitted monoterpenes. When averaged across the five most abundant genera in Amazonia, *Protium*, *Licania*, *Inga*, *Eschweilera* and *Pouteria*, isoprene remained predominant compared to monoterpenes, with 63.8% of the species emitting isoprene and 17.4% monoterpenes. These genera are widely distributed throughout the Amazon Basin and the Guiana Shield, representing an estimated 18.7% of all the arboreal individuals of the region (ter Steege et al., 2013). Thus, our results imply that the emission of volatile isoprenoid compounds could have favored the establishment and survival of these genera. This is consistent with a recent literature survey of tropical plants that reported maximum temperatures for net photosynthesis was ~1.8°C higher for isoprene-emitting species than for non-emitters, and thermal response curves were 24% wider (Taylor et al., 2019). These results led to the hypothesis that isoprene emission may be an adaptation to warmer thermal niches, and that emitting species may fare better under global warming than co-occurring non-emitting species (Taylor et al., 2019). Thus, the production of volatile isoprenoids may be important for the survival and dominance of abundant tropical genera, especially considering the high degree of abiotic stress regularly experienced in the Basin. Tropical regions receive high solar insolation due to their geographic position near the equator. Daytime leaf temperatures are high and regularly exceed 40-45 ^{o}C in the dry season (Jardine et al., 2017). While most attention has been given to the percentage of isoprenoid emitting species in the tropical biome, our study highlights the importance of quantifying their geographical distribution and absolute abundance. This quantification is key to developing improved

terrestrial land models which capture isoprenoid emissions from the biosphere. There are also important associated climate feedbacks, including modification to the lifecycles of atmospheric oxidants, aerosols, and clouds (Poeschl et al., 2010). Moreover, we suggest that volatile isoprenoids should be treated as defense compounds which protect tropical forest gross primary production under abiotic stress. They also enable a rapid recovery of net carbon assimilation mechanisms when environmental conditions improve (e.g., stomatal opening following a lowering of temperatures and rehydration of soils).

Due to the anthropogenic influence on climate, with increased emissions of greenhouse gases, surface temperatures are expected to increase and more severe, extensive, and prolonged drought events are predicted in the tropics (Field et al., 2014; Fineschi et al., 2013). Some studies have shown that tree mortality and disturbance events are increasing in tropical forests (Brienen et al., 2015). Due to the effect of isoprene and monoterpenes on atmospheric chemistry at regional and global levels and to the protection of the photosynthetic apparatus, it is of great relevance to continue to investigate the presence of light-dependent leaf emissions of isoprene and monoterpene in tropical forests, especially given their high diversity and increased pressure from land use, expansion of deforestation, and changes in precipitation regimes (Chambers and Artaxo, 2017; Harrison et al., 2013; Jardine et al., 2016a; Khanna et al., 2017).

While several hypotheses are under investigation regarding the mechanism of protection that isoprene provides during abiotic stress, an emerging view is that isoprene production and emission is tightly linked to its biosynthesis. Isoprene synthesis in the light directly consumes the products of the light reactions of photosynthesis (ATP and NADPH). Thus, isoprene production operates in parallel with other biochemical processes which consume the bulk of excess photosynthetic energy and reducing equivalents like photorespiration. This photo- and thermoprotective mechanism is supported by the

results of our light response curves. A non-linear relationship was observed between isoprenoid emissions and photosynthesis in all species and individuals studied, for which the percentage of photosynthate emitted as a volatile isoprenoid increases with light intensity as previously observed in tropical species (Jardine et al., 2016b). This result is predicted by energetic models that simulate isoprene emissions as a function of the available reducing power (NADPH) and energy (ATP) in the chloroplast (Niinemets et al., 1999). Thus, dynamic vegetation models attempting to simulate the future of forest composition and function under a changing climate should directly incorporate isoprenoid defenses. An explicit link should be included to photosynthesis for both carbon skeletons and energy/reducing equivalents.

4. Conclusions

In this study, we have shown wide-spread isoprenoid leaf emissions in the Amazon basin linked with photosynthesis with a focus on the hyperdominant tree species that account for a large fraction of all individuals. We found that four of the five most abundant genera showed a very high proportion of isoprene-emitting species. A smaller fraction had monoterpene emissions instead of isoprene. Importantly, in each of the five abundant genera at least one species was also observed to show both isoprene and monoterpene emissions with the blend of monoterpenes emitted, which can be attributed to the presence of a myrcene/ocimene synthase enzyme. As the emerging view that isoprene synthase evolved in close relation with myrcene/ocimene synthase, the results have important implications for understanding the evolution of leaf isoprene and monoterpene emissions in the tropics. The results are consistent with literature discussions of the biological functions of isoprene and monoterpene production as an important thermotolerance mechanisms which facilitate adaptation to warmer thermal niches resulting in widespread establishment of abundant tree genera in the Amazon basin.

Moreover, our findings will be useful in the development of an improved representation of terrestrial isoprenoid emissions in Earth system models. These models aim to quantitatively simulate the role of isoprenoid emissions in the terrestrial carbon cycle and atmospheric chemistry/climate feedbacks.

5. Experimental

5.1 Field sites

In this study, four different sites in the Amazon Basin were surveyed for tree species with leaf isoprene emissions between 2014 to 2016. In Amazonas State, we collected the majority of samples from the ZF-2 Tropical Forestry Experimental Station, located ~60 km at northwest of Manaus, Brazil. The vegetation is classified as undisturbed mature rainforest, with an area of approximately 230 km². We collected data from 130 trees of 89 different species in this field site. The individual and species quantities varied as some species were sampled more than once, with more than one individual. Samples were also collected in the National Institute for Amazonian Research campus, in the city of Manaus. There, we sampled 6 trees of 5 different species. In Pará State, we conducted surveys at Caxiuanã National Forest and Tapajós National Forest, both federal conservation areas. Caxiuanã is located in the municipality of Melgaço, 400 km west of the capital Belém and has an area of 3,300 km². In Caxiuanã, 9 trees of 8 species were sampled. Tapajós National Forest, with an area of 5,273 km², is near the city of Santarém at kilometer 67 on the BR-163 road. We sampled 17 individual trees of 16 species in Tapajós National Forest.

5.2 Volatile isoprenoid emissions and net photosynthesis during light response curves and under standard environmental conditions

A more detailed description of the methods employed for the simultaneous collection of leaf volatile isoprenoid emissions and gas

exchange can be found in the *MethodsX* paper, “Development of a portable leaf photosynthesis and VOC emission system” (Jardine et al, 2020b). Briefly, for all leaf samples studied for volatile isoprenoid emissions, branch cuttings were conducted in the upper canopy with sun exposed leaves with the assistance of a tree climber utilizing a pole pruner or directly accessed from flux towers. Large branches were removed from the upper canopy (up to 0.5-1.0 meter in length) and rapidly recut on the ground under water to maintain the transpiration stream. Net photosynthesis and isoprene and monoterpene emission rates were quantified from leaves during controlled changes in photosynthetically active radiation (PAR) using a commercial leaf photosynthesis system (LI-6400XT, LI-COR Inc., USA) interfaced with a gas chromatograph-mass spectrometer (GC-MS, 5975C series, Agilent Technologies, USA). A modification to the LI-6400XT was made such that a fraction of the air exiting the leaf chamber was diverted to thermal desorption (TD) tubes for the quantitative collection of any isoprene and monoterpenes emitted from the sample leaf into the chamber. TD tubes were purchased commercially, filled with quartz wool, Tenax TA, and Carboxeen 1003 adsorbents (Markes International, UK). All tubing and fittings employed downstream of the leaf chamber were constructed with PFA Teflon (Cole Parmer, USA). Hydrocarbon free ambient air was delivered to the LI-6400XT gas inlet using a capillary-grade hydrocarbon trap (Restek, USA). For all samples, the flow rate of air into the leaf chamber was maintained at 537 ml min^{-1}, the internal fan was set to the maximum speed, the leaf temperature was maintained at 30 °C, and the reference CO_2 concentration entering the chamber was maintained at 400 ppm. Using a tee fitting, air exiting the leaf chamber was delivered to the TD tube (75 ml min^{-1} when collecting) with the remainder of the flow diverted to the vent/match valve within the LI-6400XT. The excess flow entering the vent/match valve was maintained to at least 200 ml min^{-1} by loosely tightening the chamber onto the leaf using the tightening nut.

VOCs exiting the leaf chamber were collected on TD tubes for 10 min at 75 ml min^{-1} automatically during light-response curves using a portable 28 tube auto sampler (Less-P, Signature Science LLC., Austin, TX, USA), and manually during standard environmental conditions using a hand held sampling pump downstream of the TD tubes (Casella Apex Lite Pro, Casella USA, Amherst, NH, USA). For the light response curves, the sample leaf was placed in the dark chamber (0 µmol m^{-2} s^{-1} PAR), and following a 5 min period of equilibration, the sample and reference infrared gas analyzers were matched, and light curve autoprograms on both LI-6400XT and Less-P were initiated. For the LI-6400XT, the light curve autoprogram consisted of logging data every 30 seconds while controlling PAR for 10 minutes at each PAR level (0, 100, 250, 500, 1000, 2000 µmol m^{-2} s^{-1}). The autoprogram for the Less-P controlled the sequential sampling of VOCs onto 6 TD tubes, one for each PAR level. An analysis of isoprene and monoterpene concentrations from an empty chamber revealed negligible to undetectable backgrounds. Moreover, leaf isoprene and monoterpene emissions in the dark (PAR flux of 0 µmol m^{-2} s^{-1}) also showed negligible to undetectable values.

For emissions under standard environmental conditions (30 °C leaf temperature, 400 ppm reference CO_2, 1000 µmol m^{-2} s^{-1} PAR), the leaf was placed in the chamber and allowed to stabilize for up to 10-15 minutes or until stomatal conductance and net photosynthesis values stabilized. Following stabilization of the gas exchange parameters, the IRGAs were matched and isoprene and monoterpene emissions were collected together with gas exchange data for 10 minutes. For every TD sample collected with a leaf in the chamber, a second TD sample was collected without a leaf in order to demonstrate that the isoprenoid emissions derived from the leaf and not contamination of the system from one species to the next. Once collected, the TD tubes were analyzed for isoprene and monoterpene concentrations within 1-5 days using an automated Thermal Desorption – Gas Chromatography – Mass Spectrometry (TD-GC-MS)

as described below. Isoprene and monoterpene fluxes were calculated as previously described based on the flow rate of the air into the chamber, the concentration of volatile isoprenoids inside the chamber, and the leaf area inside the chamber (6 cm^2) (Jardine et al., 2014; Jardine et al., 2017).

5.3 Qualitative volatile isoprenoid emissions using dynamic enclosures

For the collection of isoprenoid emissions from palm plants in the ZF2 forest preserve in the central Amazon, we used a custom 300 mL glass leaf chamber with the inlet exposed to ambient air and the outlet connected to a TD tube with a hand held Casella pump downstream. Volatile emissions were determined qualitatively by comparing TD samples with and without a leaf in the chamber. The sampling flow used in this case was 150 mL min^{-1} for 10 minutes, for a total of 1.5 L. It should be noted that while intact leaves on the tree were studied without branch removal, this setup did not permit any control of parameters such as temperature, PAR and CO_2 concentration.

We also used an alternative type of qualitative analysis for volatile isoprenoid emission from entire branches left intact on the target tree. We placed a 5.0 L teflon bag with ¼” inlet and outlet fittings directly over a branch without sealing it (bottom open to the atmosphere). We immediately collected a 500 mL air sample onto a TD tube inserted into the enclosure outlet fitting and compared this to a 500 mL air sample collected onto a second TD tube inserted into the enclosure outlet fitting but without a branch in the enclosure. These two techniques will be referred to as qualitative 1.

Another qualitative technique consisted of the use of a high sensitivity quadrupole proton transfer reaction and mass spectrometry (PTR-MS, Ionicon Analytik, Austria) interfaced to a dynamic branch enclosure (5 L Teflon chamber) with 5 L min^{-1} of hydrocarbon free zero air flowing through as generated using a zero

air generator (Aadco 737 pure air generator). PAR at branch height was set to roughly 1,000 µmol m^{-2} s^{-1} using a grow light (90 W UFO grow light - red / blue LED light system). Isoprene was quantified at m/z 69 while monoterpenes were quantified at m/z 137. While the system was regularly calibrated for isoprene and monoterpene concentrations using a primary gas standard, we did not determine the leaf area that was placed inside the chamber from the detached branches recut under water or the temperature inside the chamber. As leaf temperature was also not determined, this method is also considered qualitative and referred to here as qualitative 2.

5.4 Thermal desorption gas chromatography-mass spectrometry (GC-MS)

Following collection of volatile isoprenoids from dynamic leaf/branch enclosures, TD tube samples were returned to the analytical laboratory in Manaus, Brazil and analyzed for monoterpenes within two days using TD-GC-MS. TD tubes were analyzed for isoprene and monoterpenes using a thermal desorption system (TD-100, Markes International) interfaced with a gas chromatograph/electron impact mass spectrometer with a triple-axis detector (5975C series, Agilent Technologies, Santa Clara, CA, USA) at INPA, Manaus, Brazil, as previously described (Jardine et al., 2017).

TD tube samples were analyzed with a TD-100 thermal desorption system (Markes International, UK) interfaced to a gas chromatograph/electron impact mass spectrometer with a triple-axis detector (5975C series, Agilent Technologies, USA). After loading a tube in the TD-GC-MS system (up to 50 analyzed sequentially), the collected samples were dried by purging for 4 minutes with 50 ml min^{-1} of ultra-high purity helium (all flow vented out of the split vent) before being transferred (290 °C for 5 min with 50 ml min^{-1} of helium) to the TD-100 cold trap (air toxics) held at 20 °C. During GC injection, the trap was heated to 290°C for 3 min while back-flushing with carrier gas at a flow of 6.0 ml min^{-1}. Simultaneously, 4.0 ml min^{-1} of

this flow was directed to the split and 2.0 ml min^{-1} was directed to the column (Agilent DB624 60 m x 0.32 mm x 1.8 µm). The oven temperature was programmed with an initial hold of 3 min at 40 °C followed by an increase to 230 °C at 6 °C min^{-1}. The mass spectrometer was configured for trace analysis with a 15 times detector gain factor and operated in scan mode (m/z 35-150).

The GC-MS was calibrated to authentic monoterpene standards (99%, Sigma Aldrich, St. Louis, MO, USA) in methanol using the dynamic solution injection (DSI) technique (Jardine et al., 2010) by dynamic dilution with a hydrocarbon free air flow of 1.0 L min^{-1}. Identification of individual monoterpenes from TD tube samples was performed by comparison of mass spectra with the U.S. National Institute of Standards and Technology (NIST) mass spectral library and by comparison of mass spectra and retention time with the authentic liquid standard which consisted of 10 µg/ml each of the following monoterpenes in methanol [alpha-pinene (CAS# 80-56-8), camphene (CAS# 79-92-5), D-limonene (CAS# 138-86-3), sabinene (CAS# 3387-41-5), 3-carene (#13466-78-9), myrcene (CAS# 123-35-3), terpinolene (CAS# 586-62-9), and trans-beta ocimene (CAS# 13877-91-3)]. Isoprene was calibrated regularly throughout the multi-year experiment by dynamic dilution of a 1.0 ppm primary standard in nitrogen as previously reported (Jardine et al., 2016b). TD-GC-MS calibrations were conducted to establish retention times and identities of sample monoterpenes, with peak area responses demonstrated to be highly linear (Jardine et al., 2017).

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7. Supporting information

The Supporting Information document contains the database from volatile isoprenoid leaf emissions and net photosynthesis in the Amazon Basin (Database_S1.xlsx) with a description of important metadata including sampling data and location, genus, species, family, tree number, research site, light intensity, identity of significant volatile isoprenoid detected, TD-GC-MS and Licor6400XT file names, exported isoprenoid emission flux file, TD-GC-MS peak areas for isoprene (m/z 67), maximum net photosynthesis and isoprenoid emission rates for light response curves, and sampling flow rate and duration. In addition, the raw data for specialists is available for download via the companion data in brief article (Jardine et al., 2020a) including the raw calibration and sample TD-GC-MS data files in Agilent Masshunter file format (http://dx.doi.org/10.15486/ngt/1602144) and raw Licor6400XT gas exchange files in MS Excel format (http://dx.doi.org/10.15486/ngt/1602143). In addition, calculated leaf isoprenoid emission rates from 47 individuals during controlled light response curves are also available (http://dx.doi.org/10.15486/ngt/1602142).

8. Figures and Tables

Authors	Region	Percentage isoprenoid emitters
Keller and Lerdau (1999)	Panama	51 species (29% isoprene)
Geron et al. (2002)	Costa Rica	20 species (50% isoprene)
Harley et al. (2004)	Brazilian Amazon	125 species (38% isoprene)
Loreto and Fineschi (2015)	Tropical and temperate forests	1,247 species (20% isoprene)
This study, Jardine et al. (2020)	Brazilian Amazon	113 species (61.9% isoprene, 15% monoterpenes, 9.7% isoprene and monoterpenes). 69 hyperdominant species (63.8% isoprene, 17.4% monoterpenes, 11.6% isoprene and monoterpenes)

Table 1: Summary of volatile isoprenoid emission surveys in tropical and temperate forests.

Figure 1: Images of the coupled leaf portable photosynthesis (Li6400XT) and volatile emission autosampler (Less-P) system developed in this study for the combined analysis of net photosynthesis and volatile isoprenoid emissions at remote field site locations in the Amazon forest.

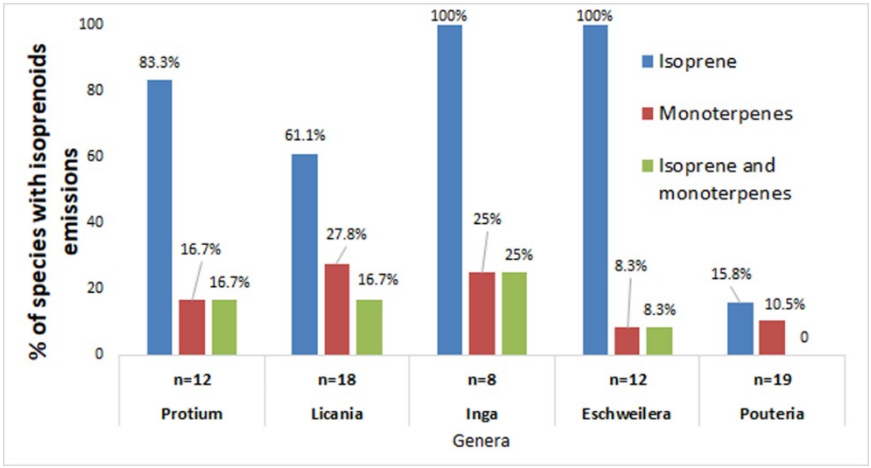

Figure 2: Percentages of hyperdominant species with leaf isoprenoid emissions for the abundant genera *Protium*, *Licania*, *Inga*, *Eschweilera* and *Pouteria* in the Amazon forest. n is the number of species sampled for each genus.

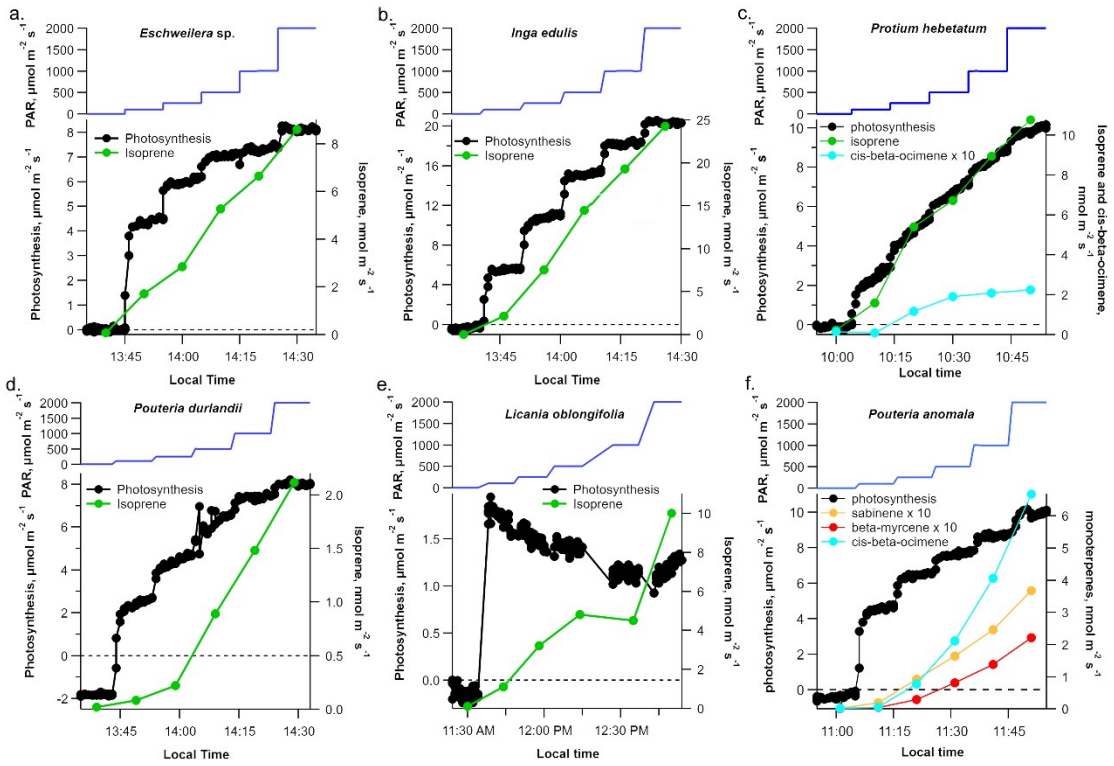

Figure 3: Example light (dark blue trace) response curves of leaf photosynthesis (black trace), isoprene emissions (green trace) and monoterpene emissions from an individual in each of the 5 abundant genera including (a) *Eschweilera* sp., (b) *Inga edulis*, (c) *Protium hebetatum*, (d) *Pouteria durlandii*, (e) *Licania oblongifolia*. Also shown is an example light response curve from the monoterpene emitting species (f) *Pouteria anomala*. The dotted line represents a net flux of zero on the photosynthesis axis with negative values in the dark due to leaf respiration. Note that (c) *Protium hebetatum* is both an isoprene and monoterpene emitter while (f) *Pouteria anomala* is a monoterpene only emitter.

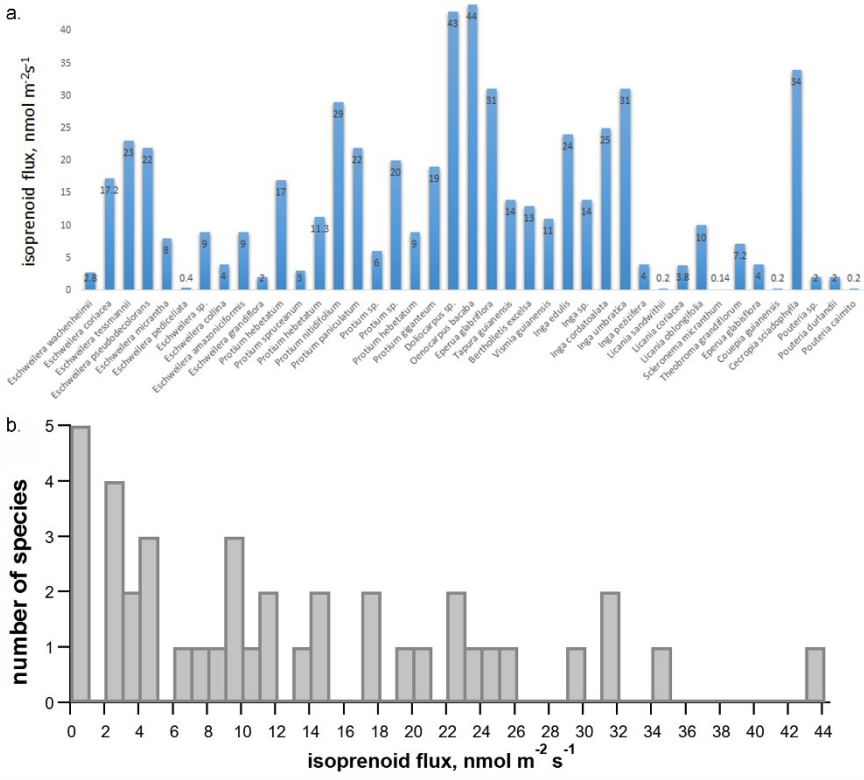

Figure 4: Maximum leaf isoprenoid emissions from the light response curve data showing (a) maximum isoprenoid emissions for species where emissions were detected and (b) a histogram representing the distribution of maximum leaf isoprenoid emissions.

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